"South African Horse-sickness: its Pathology and Methods of Protective Inoculation." By ALEXANDER EDINGTON, M.B., C.M., F.R.S.E., Director of the Colonial Bacteriological Institute, Cape Colony. Communicated by Sir DAVID GILL, F.R.S. Received August 20, 1900.

This disease, so far as is known, is peculiar to South Africa.

While affecting the Transvaal and Rhodesia every year—beginning about the end of October and continuing until the following May, or even later—it only affects the Cape Colony and Natal in an epizootic form in certain years, although sporadic cases occur annually in certain localities.

Animals Affected.—It affects horses, asses, mules, and it has been asserted—although I have never seen such cases—that quaggas have also been killed by it.

A disease which occurs to a limited extent among cattle, called by the natives Imapunga, and one which exists as a widespread plague among high-bred sheep and goats in the Eastern Province of Cape Colony, are each closely related in their pathology with this malady.

Areas Infested.—The most dangerous areas are those which are relatively low-lying—independent of the absolute altitude of the district.

Period of Infection.—It has been commonly observed that where animals during a season of sickness are not permitted to graze after sunset and before the sun has dried up the dew from the herbage, they do not so commonly become affected as where such a routine is not carried out.

Horses which are stabled during the night are, as a rule, safe, but during last year 60 per cent. of the stabled horses in Eshowe, Zululand, died of this sickness. Veterinary Lieutenant Coley, A.V.D., who kindly made the observations for me, stated that these horses were mainly fed on Guinea or Ubaaba grass mixed with forage or Indian corn. This grass was usually cut in the evenings and made into bundles till next day. Those who took particular care to have the grass thoroughly dried in the sun before using it did not lose their horses, while those neglecting this precaution lost heavily.

The disease is only directly contagious; for while inoculated horses have died in my stables among clean animals, I have never found, during observations extending over seven years, a single case of infection from such a source.

The annual mortality in Rhodesia and the low-lying parts of the Transvaal amounts to over 90 per cent.

Animals which have recovered from the sickness are termed "salted," and are from six to ten times increased in market value.

Secondary Fever.

Animals which are "salted" are liable to subsequent attacks of fever which have no necessary relation to fresh infection. I have observed numerous cases of this description among the "salted" animals under my observation and during periods when the sickness was unknown.

Symptoms of the Disease.

It occurs under two forms—the Dikkopziekte* and the Dunpaardeziekte.† In the former the head and neck swells up enormously, thus affording trustworthy indications of illness during life. In the latter form, as a rule, no symptoms appear until close to the period of death, when the animal becomes subject to very rapid breathing with heaving at the flanks. At the moment of death, in both forms, it is common to find a huge cloud of white foam ejected from the mouth and nose. This foam is produced from a free exudation of blood plasma into the air passages.

Owing to the fact that the animals suffering from the Dunpaardeziekte show no symptoms until toward the end of the period of illness, it had come to be believed that the whole period of the disease was limited to a few hours' duration.

Post-mortem Phenomena.

The pericardium is almost invariably filled with a yellow fluid which, while usually clear, is sometimes blood-stained. Solidified gelatinous exudate is frequently found in relation to the beginning of the aorta. The pleural cavity is frequently occupied by yellow fluid, and the interlobular and sub-pleural tissues are also frequently distended by this material. The interlobular tissue is frequently so distended by exudation that the lung tissue proper is dissected up in all directions. The subcutaneous tissue, especially about the great vessels in the neck, is commonly found to be invaded by this exudation, while in the Dikkopziekte the swelling of the head and neck is due to this effusion.

The yellow fluid of the pericardium and the pleuræ is spontaneously coagulable in the presence of minute traces of blood.

These represent the more characteristic pathological conditions obtaining in this disease, among which one characteristic is most noticeable by its absence, *e.g.*, inflammatory phenomena. Pathological

* Dikkopziekte, a Dutch word signifying "thick-head sickness," is applied to the form in which the swollen head is the most obvious symptom.

† Dunpaardeziekte, "thin horse-sickness," applied to the form in which the head is little or not at all swollen.

phenomena are, therefore, for the most part to be ascribed to the marvellous exudation of blood plasma, which, while seen more or less throughout the serous and subcutaneous tissues, is best marked within the thoracic cavity.

In my annual reports as Director of the Colonial Bacteriological Institute I have referred to the morbid anatomy in greater detail.

Inoculation Experiments.

For the purpose of conveying to healthy animals the infection from those already sick three materials have been made use of, viz.—

1. The yellow fluid from the trachea of infected animals.
2. The yellow fluid from the pericardium of infected animals.
3. The blood of infected animals.

The use of the first two fluids has not always been successful in setting up the disease, but fresh virulent blood has invariably proved successful.

Methods of Use of the Materials mentioned.

(*a.*) By subcutaneous injection.

(*b.*) By insertion of a seton, impregnated with the fluid, under the skin.

(*c.*) By drenching, *e.g.*, giving a dose by the mouth.

Sites Selected.

(*a.*) Directly into the lung tissue by hypodermic needle operating through the skin over an intercostal space.

(*b.*) Into the subcutaneous tissue of the neck.

(*c.*) Into the subcutaneous tissue of the flank.

(*d.*) Intravenously.

Any one of the channels selected is equally suitable, but the incubation period is somewhat shorter when the intravenous method is used.

Period of Incubation.

When the malady is induced by the inoculation of 2 or 3 c.c. of the blood of an animal which has died from spontaneous disease, a mean period of eight to nine days supervenes, after which the temperature begins to rise. The elevation is gradual, with remissions during the night, but attains to 106° F., as a rule, before death, which usually occurs after four or five days of fever.

Preservation of the Virus.

After having transmitted the disease through a succession of animals, I found it possible to preserve its virulence unimpaired through a long period of time.

I bleed the animals into bottles which hold 800 c.c. of fluid. These bottles are prepared by placing in them 50 c.c. of a 10 per cent. solution of neutral citrate of potash and plugging the necks with cotton wool. Such bottles are sterilised in the autoclave previous to use. After being filled with blood, the influence of the citrate arrests coagulation, and the corpuscular matter soon subsides, leaving the more or less clear plasma above. The latter is drawn off to half the original bulk, and is replaced by a 50 per cent. solution of glycerine and water, containing 0·25 per cent. of pure carbolic acid. Such a mixture preserves its virulence quite unimpaired for over two years. One c.c. of this material serves to induce the disease in its characteristic form, but if the dose is increased to 3 or 5 c.c., the period of incubation is shortened, and the post-mortem phenomena are less characteristic.

From the observations I have made I have found that the subcutaneous injection of fresh or properly preserved horse-sickness blood produces symptoms during life, and shows pathological changes after death which are not to be distinguished from those found in the spontaneously occurring cases of the lung form of the disease. It is somewhat remarkable that the only cases in which I have succeeded in producing the Dikkop form were those in which the virus which was used was somewhat septic. When, however, I have inoculated virulent preserved blood into partially protected animals, I have in a number of cases, although not in all, produced this form. In three cases where the virus used has been sufficiently attenuated as not to produce death but a longer febrile period than is found in the fatal cases, I have also seen the Dikkop form produced.

Effect of Desiccation on the Virus.

Citrated blood dried in a thick layer was rendered non-virulent. Where, however, such blood was rapidly dried on glass plates in very thin layers it was found, when 2 grammes was dissolved in salt solution and injected into horses, that it produced fever, but not in a virulent form. The fever thus induced gave practically no protection against a dose of virulent blood at a subsequent date. Mild attacks of horse-sickness do not, however, give such protection as is required to resist virulent blood.

Experiments made in regard to Protective Inoculation.

The yellow fluid from the pericardium of an animal which had died from the disease was filtered through a Pasteur filter. 100 c.c. of the filtrate was injected subcutaneously into a horse. Eleven days later it was inoculated with 3 c.c. of preserved blood injected subcutaneously. The result, which culminated in death from ordinary horse-sickness, showed that no protective influence had been exerted by the filtered fluid.

Effect of Calomel.—This drug, in doses of 30 to 60 grains daily, had the effect of retarding death, and the blood of such animals drawn at periods later than that at which death usually occurs was distinctly weakened in virulence. Such blood has on several occasions, though not in all, induced attacks of the disease, from which the animals not only recovered, but acquired protection against virulent blood injected subsequently.

Transference of the Disease to other Animals.

(*a.*) *Donkeys.*—The subcutaneous or intravenous inoculation of donkeys with fresh virulent blood is followed by fever. The period of onset is irregular and uncertain, while the duration of the febrile period varies from one or two days to, in my experience, a week or more.

The amount of the virus used has some relation to the severity of the fever, but the special susceptibility of the animal is the principal factor in determining the degree and duration of the fever.

Two donkeys, equal in age, were inoculated respectively with 1 c.c. and 50 c.c. of the same blood. In both cases a moderately severe reaction followed, and while the animal receiving the injection of 50 c.c. was rather more severely affected than the other, the difference on the whole was but slight.

In all I have inoculated twelve donkeys, and, while none died, the difference in susceptibility was most clearly demonstrated, some scarcely showing any reaction at all.

(*b.*) *Cattle.*—The susceptibility of cattle to the inoculated disease is excessively variable. I have inoculated twenty-one cattle. A definite febrile reaction was produced in seven cases, and four died.

In the case of one which died, and in which the symptoms produced were quite characteristic of those found in horses, the inoculation of its blood into a clean horse was followed by the usual period of incubation, the onset of fever, and death from characteristic horse-sickness.

The disease known as Imapunga, which occurs to a limited extent among cattle, presents features which in every respect are identical with those produced in susceptible cattle by the inoculation of virulent horse-sickness blood.

(*c.*) *Goats.*—Angora goats are also to a limited extent susceptible to horse-sickness infection. Of seventeen which were inoculated, a febrile reaction occurred in ten; none died. From one of the ten blood was taken, which was used to inoculate an ox. The latter animal developed fever, and died with exactly similar symptoms during life, and showed the same post-mortem conditions as the ox already referred to, whose blood, when inoculated into a horse, produced characteristic horse-sickness.

(*d.*) *Sheep.*—Sheep are also susceptible. Of ten which were inoculated, six showed a well-marked febrile reaction, but none died.

I have not succeeded in transferring the disease to rabbits, guinea-pigs, rats, or mice.

The Transmission of the Disease for Protective Inoculation by Means of the Inoculation of Fresh Blood.

The inoculation of horses with the blood of donkeys which were suffering from fever produced by inoculation has been attended with most varying results.

In some cases death has been produced, in some an irregular febrile period, while in others no apparent result has followed. The period of onset of the fever has likewise been most variable. In some cases a reaction has been set up corresponding to the normal period of incubation which obtains in horse-sickness, while in other cases reaction has been delayed for more than 25 days.

Influence of the Reaction produced.

Where fever has set in on or about the eighth day, been moderately severe in degree and duration, and subsequently subsided, a very definite degree of protection has been produced, although seldom high enough to set up such a resistance as to oppose death when the animal was subsequently inoculated with virulent blood.

A striking demonstration of variable susceptibility among horses was furnished during these experiments. Of three horses and one mule which were each inoculated subcutaneously with 5 c.c. of fresh blood—

The mule had no reaction.
Two horses had scarcely any reaction.
One horse had a good reaction.

In the case of the last horse, when subsequently inoculated with virulent blood it suffered severely and just managed to recover. The others had not been protected to any appreciable degree. Obviously, therefore, the susceptibility of the last animal had been such as to admit of infection from the donkey's blood producing reaction and the

establishment of protection, whereas the higher degree of insusceptibility of the other animals resisted infection, and in this way evaded the onset of protection. This phenomenon forms the greatest barrier to protective inoculation, and has contributed to the enormous trouble I have experienced in devising a practical method of protective inoculation.

The fresh infected blood of cattle, sheep, and goats is still more variable in its results than that obtained from the donkey.

Numerous other experiments of the same nature have been made, all of which result in showing—

(*a.*) That donkeys, oxen, goats, and sheep possess a very irregular susceptibility to the disease.
(*b.*) That the blood of donkeys which do not react may produce no effect when inoculated into the horse.
(*c.*) That the blood of donkeys which have evinced moderate reaction may produce intense reaction in some horses and practically none in others.
(*d.*) That a mild reaction in the donkey furnishes no definite assurance as regards the reaction which its blood may set up in horses.

Owing to the variable quality of the infection possessed by infected donkey's blood in the fresh state, I experimented with blood taken from donkeys and oxen which, after having been received, was preserved in the manner already described.

A large number of experiments carried out by this means furnished the following results:—

1. Protection was only obtained where a definite amount of fever had been produced on several occasions, but unless the reaction was severe, the animal did not resist the inoculation of 1 c.c. of preserved virulent blood at a later period.
2. The susceptibility of horses to such a weakened or attenuated virus varies enormously. Of two animals inoculated with the same dose of the same virus injected directly into the jugular vein, one had good reaction, the other very feeble. Neither were found to be protected when subsequently inoculated with virulent blood.

One inoculated with the same amount of the same preserved material two months later died from the primary inoculation, thus showing that even the attenuated virus can be satisfactorily preserved for a considerable period of time.

In the case of another animal which was inoculated intravenously with this virus no result followed. Fifteen days later the same inocu-

lation was repeated. The temperature began to be elevated on the fourteenth day, and it died of horse-sickness seven days later. The primary inoculation in this case, while being ineffectual to induce the disease, had evidently lowered the susceptibility, so that a fresh stimulus, by the same virus, was sufficient to overcome the resistance entirely.

Having recognised that the blood of animals which lived beyond the ordinary period at which horses usually die from horse-sickness was lowered in virulence, I determined to attempt to produce this change *in vitro*.

Having, therefore, prepared bottles containing citrate solution, and having thoroughly sterilised them, selected animals were bled under the most rigid aseptic management, and the blood received in the bottles which, after being replugged, were incubated at a temperature of 102 F. during ten days. In one such experiment, out of a total of fourteen bottles, thirteen remained perfectly free from extraneous organisms. Such blood after incubation was then preserved and tested.

I found, in this manner, that it was possible to produce an attenuated virus equally suitable for inoculation as that obtained from the donkey or the ox.

While, however, these experiments demonstrated that it was possible to protect horses by repeated inoculations of an attenuated virus, they equally demonstrated the irregularity of action, owing to the varying susceptibility to the disease in its attenuated form which obtains among horses.

Several important facts, however, which were elucidated, are deserving of careful consideration, viz. :—

Death in cases of horse-sickness cannot directly be ascribed to hyperpyrexia, inasmuch as several horses have recovered after having experienced temperatures of over 107 F.; while others, which have died, and, in which characteristic lesions have been found, have not had a temperature exceeding 105 F.

Protection can be arrived at without the production of very great reaction, provided that a number of inoculations are made into the animal, and that these have been so arranged as to proceed very gradually to the highest degree of virulence.

3. It is exceedingly difficult to determine the exact degree of attenuation in any particular sample of an attenuated virus. I have usually attempted this by the inoculation of the virus into one or, at most, two horses; but if the susceptibility of such animals happens to be of a low grade, then the reaction produced may not obtain in other horses for which it may subsequently be used. In other words, to determine

exactly the strength of an attenuated virus, it would be necessary always to make the test on at least five animals.

4. The indication for future experimentation was thus to call for the discovery of some method by which a virus of standard virulence might be, at will, reduced to any required degree of attenuation.

Experiments were also made to determine whether the blood of an animal suffering from "secondary" fever had any infective property. To this end animals under "secondary" fever, with temperature as high as 106 F., were bled and the blood used to inoculate clean animals, but in no case was any reaction produced thereby. I therefore am convinced that the blood during "secondary" fever is non-infective.

Experiments with Serum and Defibrinated Blood of Animals which have recovered from Horse-sickness.

The experiments made have included serum derived from—

1. Animals formerly "salted."
2. Animals formerly "salted" and subsequently reinoculated by periodic injections of gradually increasing doses of virulent blood, the maximum dose being 1000 c.c.
3. Animals treated as in Clause 2, but subsequently permitted to rest for several months and then reinoculated with a small dose (5 c.c.) of virulent blood.

Under the first clause, serum was obtained from a well-"salted" animal which had been twelve days, previous to bleeding, inoculated with 5 c.c. of preserved virulent blood.

Animals which were inoculated with 2 c.c. of virulent blood were subsequently inoculated with large doses of serum (100 c.c. or more). The inoculations, in some cases, began on the day that virulent blood was injected; in others it was delayed until the temperature began to rise, but although the total amounts given exceeded 1000 c.c., no definite interference with the course of the disease was noticeable. Under Clause 2, "salted" animals were inoculated with progressively increasing doses of virulent blood. When these animals had been inoculated with doses of virulent blood equal to 1000 c.c. they were allowed to rest for eight to twelve days, after which they were bled.

Of this serum, 500 c.c. was inoculated at one dose into a horse, which, during thirty-three subsequent days, manifested no signs of illness due to the inoculation. When this period was completed, it was inoculated with virulent virus and as a result died of characteristic horse-sickness. No evidence was shown that the serum had in any

way interfered with the action of the virus. Where, however, this serum was used to inoculate animals which were already infected, a very curious change in the character of the disease occurred.

The animals became affected, usually in thirty-six hours, with hæmoglobinuria, which later passed into hæmaturia and ended invariably fatally, if the disease was virulent. In two cases, however, where the disease had been induced by an attenuated virus, the hæmaturia came to an end with the subsidence of the fever. In all, this curious condition was produced by serum in nineteen cases.

Where animals are bled into citrate solution, the plasma is of a yellow colour, but in cases which eventually became the subjects of hæmaturia, I noticed, if they were bled about twenty-four hours previous to the onset of this condition, that the plasma was red coloured. It is therefore evident that the condition has its origin in the blood.

In several cases animals, which were partially protected, became subject in a slighter degree to this complication, if they were reinoculated with virulent blood and were unable to resist it.

This blackwater may have some relation to the blackwater fever in man. It is generally believed in Rhodesia that blackwater does not occur as a primary disorder, but only supervenes in persons who have previously been the victims of malarial fever.

It seemed to me that this serum might in some way be associated with a residual infection. To determine this I inoculated a "salted" horse, which had also had repeated large injections of virulent blood, with 50 c.c. of fresh blood. I bled it eight days later, and with 5 c.c. of its blood inoculated a clean animal, which thereafter had a very slight rise of temperature on the eighth day.

An animal similarly treated was finally inoculated with 300 c.c. injected intravenously and 20 c.c. subcutaneously thirty-nine days previous to being bled. When bled, the blood was defibrinated, and 100 c.c. was injected into each one of six animals. No evidence was shown of any infectivity of the blood.

I now determined to make use of the serum from animals which, under Clause 3, had been allowed to rest for periods over one month previous to the collection of their serum.

This serum is that which is now being used for the purpose of protective inoculation.

I have determined with regard to it—

1. It possesses no curative action which in practice would be of any avail to restrain the course of the disease.

2. Since an injection of 100 c.c., into one animal has absolutely no effect in restraining the action of 1 c.c. of ordinary preserved virus inoculated subcutaneously on the other side, it does not possess any immunising power which would be of practical value in withstanding infection.

3. Its germicidal activity is extremely weak, as is shown by the following experiments:—

(*a.*) 1 c.c. of fresh virulent blood was mixed with 100 c.c. of serum, and after being kept for twenty-four hours in the ice chest was inoculated into a clean horse. The animal had a sharp febrile reaction.

(*b.*) Another animal was treated in the same way, but the serum and blood was injected immediately after being mixed. This animal also had a reaction, but less severe than the former. Variation of susceptibility must of course be taken into account, and, in order to establish this conclusion satisfactorily, a considerable number of animals would require to have been simultaneously dealt with.

(*c.*) Equal volumes of serum and preserved blood were mixed and kept at ordinary room temperature for four days. Of this mixture, 2·5 c.c. was injected subcutaneously into a clean animal. Fever set in after the usual period of incubation, pursued its characteristic course, and the animal died under circumstances and in the usual time which obtains after the use of pure virulent blood.

Since 1 c.c. of virulent blood mixed with 100 c.c. of serum produced a sharp febrile reaction in one animal but had practically no effect in some others, and since 1 c.c. of blood and 200 c.c. of serum produced a reaction in another animal, it was clear that under this method also I should have to meet differences of animal susceptibility.

It was so far fortunate that preserved virulent blood acted equally well as fresh blood, so that a standard virus is easily prepared and maintained, and by mixing the serum of a considerable number of animals I am able to standardise a large volume of serum.

I concluded, therefore, to determine the amount of serum which, when mixed with a definite amount of blood, would serve, acting in concert with the natural protective bodies in the system of the average horse, to ensure the production of the modified disease. After fourteen days should have elapsed subsequent to this inoculation, provided a severe reaction was not set up, I intended to re-inoculate with the same dose of virulent blood, but with a much reduced quantity of serum. Again, after fourteen days, the procedure should be repeated, the dose of virulent blood remaining a constant quantity, but the dose of serum being still further reduced. Finally I intended to inoculate with virulent blood by itself.

In the first three series of experiments sixteen horses were used. These were inoculated as follows:—

1st Inoculation 1 c.c. virus and 100 c.c. serum (10 animals).
1 c.c. „ 90 c.c. „ (4 animals).
0·5 c.c. „ 50 c.c. „ (2 animals).
2nd Inoculation 0·5 c.c. virus in 30 c.c. „
3rd Inoculation 0·5 c.c. „ 15 c.c. „
} A slight variation of the quantities was made in several cases.
4th Inoculation 0·5 c.c. pure preserved virulent blood.

The following shows the results obtained, and where the remark "salted" is made, it is to be understood that the animal has, at later dates, withstood enormous doses of the most virulent blood.

Animal.	Reaction.	Result.
1st.	No reaction at all	Salted.
2nd.	Reaction to 1st only.....	„
3rd.	No reaction	„
4th.	Slight reaction after all four	„
5th.	Reaction to 4th	Died.
6th.	Slight reaction to 1st	„
7th.	No reaction	Salted.
8th.	Reaction to 4th	Died.
9th.	Slight reaction to 3rd; after 5th...	„
10th.	Slight reaction to 4th	Salted.
11th.	Slight reaction to 4th	„
12th.	Reaction to 5th.....	Died.
13th.	No reaction	Salted.
14th.	Reaction to 5th.....	„
15th.	Reaction to 4th.....	„
16th.	Reaction to 4th.....	„

In the next experiment seven animals were used, which were inoculated as follows:—

Inoculations.
1st. 1 c.c. virus and 100 c.c. serum.
2nd. 0·5 c.c. „ 25 c.c. „
3rd. 0·5 c.c. „ 10 c.c. „
4th. 0·5 c.c. „ 1·5 c.c. „ 2 had 0·5 c.c. pure virus.

The results were as follows:—

Animal.	Reaction.	Result.
1st.	Slight reaction to 5th	Salted.
2nd.	„ „ 3rd and 5th.	„
3rd.	No „ 4th	After a large dose of pure virus, died.

Animal.	Reaction.			Result.
4th.	Slight reaction to	2nd	.....	Salted.
5th.	No	„ 4th	.....	After a large dose of pure virus, died.
6th.	Slight	„ 2nd	.....	Salted.
7th.	„	„ 3rd	.....	„

Note.—Where "5th" is mentioned, it refers to a dose of pure virulent blood.

Total animals inoculated	.....	23
„ „	died.....	9
„ „	salted	14

The tests thus applied have been of the most severe character, and despite the fact that these are only of the value of preliminary experiments, the results are extremely satisfactory.

Obviously animals have been sacrificed which, under altered methods, might have been saved, for the outcome of these inoculations goes to show that, unless some reaction has been produced during the earlier reactions, there is no certainty that an animal is protected. Nevertheless it is equally proved that some have become highly protected without having shown any reaction at all.

The indication, therefore, has been to increase the dose of the virus used in the primary inoculations, even at some risk to the more susceptible animals.

In a subsequent series of animals this has been carried out in the following manner:—

1st.	Inoculation	2 c.c.	virus and	50 c.c.	serum.
2nd.	„	2 c.c.	„	20 c.c.	„

Twelve animals have been simultaneously inoculated in this manner. The reactions produced have been as follows:—

Animal.	1st Inoculation.	2nd Inoculation.
1	None.	None.
2	Slight.	Slight.
3	„	„
4	„	Severe.
5	Slight.	None.
6	„	„
7	Severe.	Slight.
8	Slight.	„
9	None.	„
10	Slight.	„
11	„	None.
12	„	Severe.

Since one animal, after the first inoculation, had a severe reaction, it is evident that the limit of strength, consistent with safety, had been reached. The reactions, in the two cases, after the second, were extremely severe, and indicate that the limit of strength of virus for that inoculation had been slightly exceeded, if a widespread scheme of operation had been intended to be carried out among animals in the open.

These results would seem to indicate that fortified serum, *e.g.*, that obtained from animals which, after "salting," have been reinoculated with large doses of virus, exerts a peculiar and definite action on the virus.

While, however, 100 c.c. of serum suffices to prevent 1 c.c. of virulent blood, when mixed with it, producing any great elevation of temperature, I have referred to a case in which a severe reaction was produced. Since, in another case, 200 c.c. of the same serum, with an equal amount of virulent blood, was followed by a reaction and a definite amount of protection, it is evident that the difference in susceptibility between the latter animal and those which react slightly after 100 c.c. of serum and 1 c.c. of virulent blood is equal to 100 c.c. of fortified serum. Moreover, as already shown, when the virus is attenuated by its passage through less susceptible animals, such as the donkey or cow, its effect, when used in the same dose, either by subcutaneous or intravenous injection, varies very greatly in different animals; in some producing no evident reaction, in others setting up some fever; while, again in others, its use was followed by the onset of the virulent disease resulting in death.

If, therefore, the admixture of serum with virulent blood is followed, on inoculation, merely by a modified form of the disease, it must be concluded that the serum, of itself, cannot be credited with this result, but that a peculiar quality, existing in the animal body, and varying in amount from animal to animal, must play an important part. Whether this principle is a simple body, or is a combination of several, cannot at this moment be determined, but for convenience' sake I would suggest that the name "Antagones" should be applied to it. The term need not be taken to imply either an antitoxic or germicidal body, but merely to denote the "defensive" properties which are already existent to a greater or less degree in all animals, or are produced or increased under special stimulation.

Since thoroughly "salted" animals and donkeys can be reinoculated and infection proved to exist in their blood for at least ten days subsequent, I am inclined to look upon the protection existing in "salted" animals as of the nature of a "tolerance," and to believe that true immunity, in horses, against this disease is never acquired.